OUTLINE OF THE PROPOSED WORK FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN PHARMACEUTICAL SCIENCES

A SYNOPSIS

"Design, Synthesis and Biological Evaluation of Some New Chemical Entities as Potential Antimalarial Agents"

Submitted for the consideration of

Registration for the Degree of *Doctor of Philosophy*

In the Faculty of Pharmaceutical Sciences and Technology,

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Design, Synthesis and Biological Evaluation of Some New Chemical Entities as Potential Antimalarial Agents.

1. AIM & OBJECTIVES:

The present study aims to Design, Synthesis and Biological evaluation of some new chemical entities as potential antimalarial agents with below mentioned objectives.

- To perform Structure-Based Drug Designing Approach with the help of the sophisticated software for docking studies and using the results of docking studies, synthesize a series of such analogues with different novel chemical classes.
- To synthesize, isolate and purify the hybrid structures of precursor and intermediates and final derivative given in the mentioned schemes.
- To characterize the synthesized derivatives individually using various advanced analytical techniques.
- To evaluate the synthesized analogs biologically to measure its biological effect on selected species of malaria.
- To perform acute toxicity studies with the help of Complete Blood Count.
- To study the data generated by various above mentioned procedures and techniques and to conclude it.

2. INTRODUCTION:

Malaria, the most threatening parasitic infection in human has been a real concern for centuries. 300 to 500 million new clinical cases are reported by the World Health Organization (WHO) every year, resulting in annual deaths of about one million people. According to a WHO report, malaria accounted for 214 million cases and an estimated 438 000 deaths worldwide in 2015. Most cases in 2015 are estimated to occur in the WHO African Region (88%), followed by the WHO South-East Asia Region (10%) and the WHO Eastern Mediterranean Region (2%). Similarly, it is estimated that in 2015 most deaths (90%) were in the WHO African Region, followed by the WHO South-East Asia Region (7%) and the WHO Eastern Mediterranean Region (2%). There were large reductions in the number of malaria cases and deaths between 2000 and 2015. The incidence rate of malaria, which takes into account population growth, is estimated to have decreased by 37% globally between 2000 and 2015; in the same period, the estimated malaria mortality rate decreased by 60%.[1]

Though malaria mortality rates have fallen during the last decade, mortality figures are still very high for a disease that is largely preventable and treatable. A major contributor to the problem is the

emergence and spread of resistance towards most drugs in clinical use such as chloroquine, amodiaquine, pamaquine and mefloquine. If new drugs are not introduced onto the market, the spread of malaria could have disastrous consequences.[2]

Malaria is the most important infectious disease caused by *Plasmodium falciparum* of anopheles mosquitoes. Four Plasmodium species with distinct disease pattern in humans exist: *P. falciparum* (malaria tropica), *P. vivax*, *P. ovale* (both malaria tertiana), and *P. malariae* (malaria quartana). P. falciparum and P. vivax account for 95% of all malaria infections. Almost all severe and fatal cases are caused by *P. falciparum*.[3]

Plasmodium falciparum dihydrofolate reductase thymidylate synthase (*Pf DHFR-TS*) is an important target of antimalarial drugs. Antifolate antimalarials such as pyrimethamine, and proguanil, the prodrug of cycloguanil , have long been used clinically in the treatmet of malaria infection, especially due to *Plasmodium falciparum*. However resistance to these inhibitory drugs emerged soon after their introduction. Hence there is still need to developed newer antifolate antimalarial.[4]

4-aminoquinoline, the nucleus of CQ and triazine which is the nucleus of cycloguanil when joined together with a linker meets the entire structural requirement such as the presence of a hydrophobic tail and hydrogen bond donor head group respectively for inhibition of *pf-DHFR-TS*.[5] Recently, conjugates of 4-aminoquinoline and 1,3,5-triazine have been widely studied as novel *Pf-DHFR-TS* inhibitors.[6,7,8,9,10]

Chloroquine (4 aminoquinoline) can be considered to be one of the most important synthetic chemotherapeutic agents in history. Since its discovery, chloroquine has proved to be a highly effective, safe, and well-tolerated drug for both treatment and prophylaxis of malaria. Chloroquine is a potent blood schizonticide, effective against the erythrocytic forms of all strains of Plasmodia. The major concern in the treatment of malaria at present is the increasing resistance of the malarial parasite Plasmodium falciparum to antimalarial drugs, particularly chloroquine. Hence there is still need of new antimalarial drugs.[11]

3. LITERATURE REVIEW:

The extensive literature survey revealed the synthetic work on the 4- aminoquinoline

Supriya Sahu et al (2015) reported existing antifolate antimalarial drugs have shown resistance due to the mutations at some amino acids position of *Plasmodium falciparam* DHFR-TS. The present study shown synthesis and screening of some hydrid 4 -aminoquiolines triazine derivatives against pf-DHFR –TS.

Deepak Kumar et al (2015) synthesized a series of novel 4- aminoquinolinees- pyrimidine hybrids and evaluated for their antimalarial activity.

Sunny Manohar et al (2011) synthesized a series of 4- aminoquinoline- 1,2,3- triazol and 4-aminoquinoline- 1,2,3- triazol- 1,3,5- triazine hybrids and evaluated their antimalarial acivity against D6 and W2 strains of Plasmodium falciparam.

Nicolli Bellotti de Souza (2011) synthesized a new series of 4-amino-7- chloroquinoline derivatives using 4,7-dichloroquinoline linked together with a series of diaminealkyne and diaminedialkyne. These fragments are considered able to interact with membrane lipids, also to be transported into the cytoplasm and possibly, to interfere with the lipid or polyamine transport or metabolism of the parasite. They have also obtained platinum (II) complexes from these derivatives. Metal complexes have attracted considerable attention in recent years and can be used as a strategy to enhance biological activity and overcome resistance.

Raja Solomon (2007) A new series of side-chain modified 4-aminoquinolines have been synthesized and found active against *P. falciparum* in vitro and *P. yoelli* in vivo These analogs form a complex with hematin and inhibit the *â*-hematin formation, suggesting that this class of compounds act on a heme polymerization target

Sandra Gemma,(2012) synthesized and optimized 4 aminoquinoline / clotrimazole Based Hybrid as Antimalarials:

4. RATIONALE:

For several decades, the gold standard for the treatment of malaria was CQ, a 4-aminoquinoline that was previously characterized by its efficacy, low toxicity and affordability. CQ acts by binding to haem moieties produced from proteolytically processed haemoglobin inside infected erythrocytes, thereby interfering with haem detoxification. Massive worldwide use of CQ, beginning in the late 1940s, was followed a decade later by the first reports of CQ-resistant strains of *Plasmodium falciparum* antimalarial.[12]

Plasmodium falciparum had developed resistance against chloroquine and it has been one of the major drawback in malarial therapy. To overcome this problem, plethora of work has been carried out on this nucleus and SAR study shows that quinoline nucleus with chloro group at seventh position, basic side chain at terminal position is important for antimalarial activity. Further study also proved that modification of this basic ring side chain can lead to improved anti malarial activity against drug resistant *Plasmodium falciparam* strains.[13]

Further to combat drug resistant problems, concept of multi targeted hybrid drugs has been explored. In this approach two or more different target selective pharmacophore are linked covalently resulting into one molecule. These dual drug hybrids have the potential to increase bio pharmaceutical efficacy, reduce cost, decrease risk of drug- drug interaction and overcome rapid development of resistance problems. With these thought various research groups have synthesized a huge number of hybrid molecules by the combination of chloroquine with other pharmacophore acting on different targets.[2]

Amongst the currently used clinical antimalarial drugs, the antifolates have the best defined molecular targets: enzymes dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS), functioning in the folate metabolic pathway. Folate metabolism is important for the viability of malaria parasites. These two pathways have been targeted in both treatment and prophylaxis of the disease. The most widely used antifolate antimalarial drugs include pyrimethamine (PYR), proguanil, sulfadoxine (SDX) and dapsone, which have long provided chemotherapy at an affordable price to the poorer nations. 4-aminoquinoline, the nucleus of CQ and triazine which is the nucleus of cycloguanil when joined together with a linker meets the entire structural requirement such as the presence of a hydrophobic tail and hydrogen bond donor head group respectively for inhibition of *pf*-DHFR-TS. Recently, conjugates of 4-aminoquinoline and 1,3,5-triazine have been widely studied as novel *pf*-DHFR-TS

inhibitors. [14] As a part of our ongoing research work to develop hybrid antimalarial molecules, we have designed a new series of hybrid 4-aminoquinolne-pyridine derivatives.

5. PLAN OF WORK:

5.1 **Computer Aided Drug Designing:** Computer Aided Drug Design of some novel analogs of 4-Aminoquinoline as Pf-DHFR-TS antagonists. Some methods of Direct drug design such as Receptor surface mapping, Molecular docking, and Indirect drug design as Pharmacophore (ligand) mapping is planned for the designing of novel ligands for Pf-DHFR-TS, containing 4-Aminoquinoline as scaffold.

The X-ray crystallographic structure of Plasmodium falciparum dihydrofolate reductase thymidylate synthase (Pf DHFR-TS) is available on the Brookhaven's Protein Data Bank (www.rcsb.org) with PDB id *1JK3*.[15]

The various steps involved in the complete software studies are as follows

5.1.1 Design of novel Pf DHFR-TS antagonists

5.1.1. A .Receptor Surface Mapping

5.1.1 A.i Site Finding

5.1.1A.ii Site Mapping

5.1.1. B. Molecular Docking

- Ligand preparation:
- Protein structure
- Protein preparation
- Receptor Grid Generation
- Ligand Docking

5.1.1.C Pharmacophore Mapping

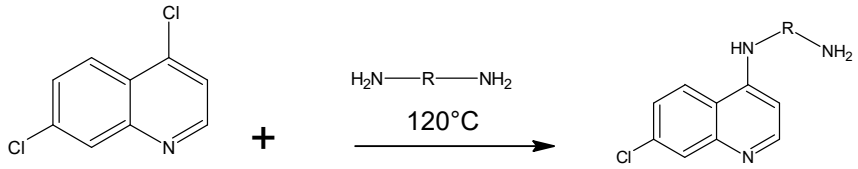
5.2 Synthesis:

5.2.1 Scheme-I

5.2.1.1 General Procedure for Synthesis of 4-Amino substituted 7-chloroquinoline intermediate:

The compound was prepared by adding methylenediamine/ethylenediamine /Diaminobenzene / diaminopropane to melted 4,7-dichloroquinoline. This was first heated at 80°C for one hour and

then refluxed at 120-130°C for 2-3 hours. The reaction mixture was then cooled to room temperature over a long period of time. It was then dissolved in dichloromethane and cold water was added to it. Brine solution was also added. The mixture was stirred with a glass rod and kept overnight without disturbance for settling the solid product in between the two solvent layers (colour of the solution changes from yellow to dark pink over night).The solid product was separated out by filtration. It was then dried in hot air oven at 45°C. The dried powder was then dissolved in n-butanol and filtered. The filtrate was poured in a petridish and n-butanol was evaporated. After complete evaporation of the solvent, pale yellow crystals appeared.

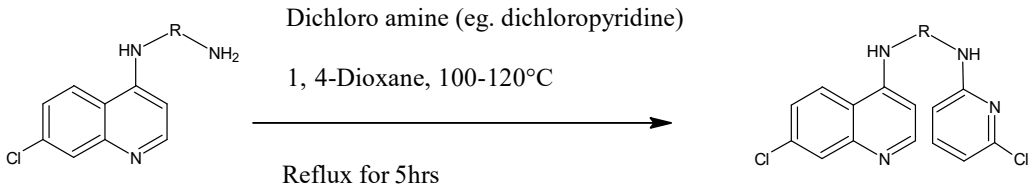


4,7-dichloroquinoline

4-Amino substituted 7-chloroquinoline intermediate

5.2.1.2 Reaction Of 4-Amino substituted 7-chloroquinoline intermediate with dichloro amine:

Dichloro Amine (eg. 2,6-Dichloro Pyridine) was dissolved in quantity sufficient 1, 4-Dioxane and the temperature was raised to about 100-120°C. To this equimolar quantity of 4-N-substituted chloroquine analogs was added and the solution was refluxed for 5 hrs. The reaction mixture was then kept overnight for solvent evaporation. Dried product was collected after complete removal of the solvent.



4-Amino substituted 7-chloroquinoline intermediate

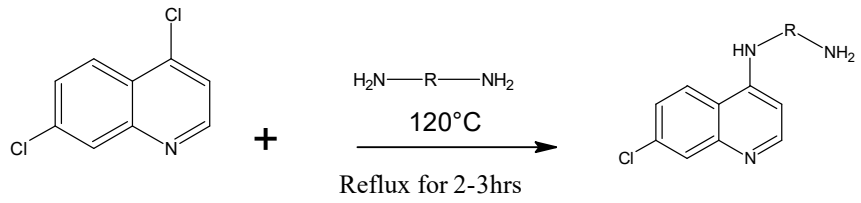
Chloropyridine substituted 4-amino chloroquine intermediates

5.2.1.3 Synthesis of Final Compounds: The Chloropyridine substituted 4-amino chloroquine intermediates were further undergo treatment with various amines to get final novel analogs of 4-aminoquinolines.

5.2.2. Scheme-II

5.2.2.1 General Procedure for Synthesis of 4-Amino substituted 7-chloroquinoline intermediate

The compound was prepared by adding methylenediamine/ethylenediamine /Diaminobenzene / diaminopropane to melted 4,7-dichloroquinoline. This was first heated at 80°C for one hour and then refluxed at 120-130°C for 2-3 hours. The reaction mixture was then cooled to room temperature over a long period of time. It was then dissolved in dichloromethane and cold water was added to it. Brine solution was also added. The mixture was stirred with a glass rod and kept overnight without disturbance for settling the solid product in between the two solvent layers (colour of the solution changes from yellow to dark pink over night).The solid product was separated out by filtration. It was then dried in hot air oven at 45°C. The dried powder was then dissolved in n-butanol and filtered. The filtrate was poured in a petridish and n-butanol was evaporated. After complete evaporation of the solvent, pale yellow crystals appeared.



4,7-dichloroquinoline

4-Amino substituted 7-chloroquinoline intermediate

5.2.2.2 Synthesis of hybrids of 4-Amino substituted 7-chloroquinoline as a final compound:

The various hybrid structures of 4-Amino substituted 7-chloroquinoline intermediate were obtained as a final compounds by removal of hydrogen from primary end nitrogen by various reactions like Schiff Base formation/ Sulfonation/Cyclization by urea/thiourea/Formation of Chalcones or Reaction with 4-Chloro-2-aminobenzothiazole.

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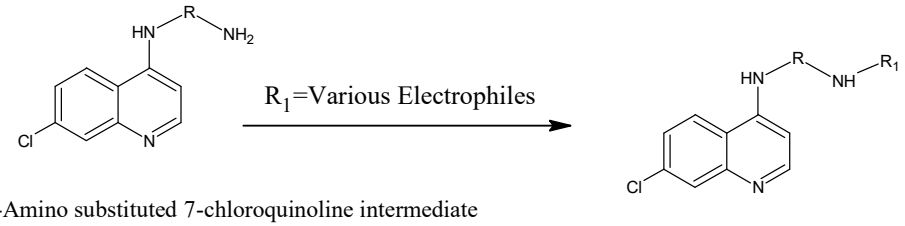

4-Amino substituted 7-chloroquinoline intermediate

Final compounds

5.3 Characterization:

The reactions will monitored by TLC. The purity of all synthesized compounds will confirmed on the basis of chromatographic and physicochemical properties. Physicochemical and spectroscopical characterization of all synthesized compounds will be assess by their melting points, R_f values determined by Thin Layer Chromatography, Infra Red (IR), ^{1}HNuclear Magnetic Resonance (^{1}HNMR), ^{13}C NMR, Mass spectrum and elemental analysis.

5.3 Biological Evaluation:

The *in vitro* anitimalarial screening of all the synthesized compounds will be perform using suitable strain of *Plasmodium Falciparum*. The synthesized new chemical entities would be screened for their possible acute toxicity using animal models.

6 DURATION OF PROJECT:

- Structure-Based Drug Designing Approach with the help of the sophisticated software for docking studies and using the results of docking studies, synthesizes a series of such analogues with different novel chemical classes.
- Synthesis, isolation and purification of the hybrid structures of precursor and intermediates given in the mentioned schemes.
- Characterization of the synthesized derivatives individually using various sophisticated and advanced analytical techniques.
- *In vitro* antimalarial screening of synthesized analogs to measure its biological effect on selected species of malaria.

Design, Synthesis and Biological Evaluation of Some New Chemical Entities as Potential Antimalarial Agents.

- To study the data generated by various above mentioned procedures and techniques and to conclude it.

Sr. No.	Activity	Tentative Duration (months)
1	Literature Review.	03
2	Selection and procurement of drugs and chemicals.	03
3	Structure-Based Drug Designing Approach with the help of the sophisticated software for docking and pharmacophore studies.	06
4	Synthesis, isolation and purification of the hybrid structures of final compounds given in the mentioned schemes	12
5	*In vitro* antimalarial screening of synthesized analogs	06
6	Compilation, interpretation of data and writing of thesis.	06
	Total	36

7 RESEARCH OUTCOME:

The current literature shows us the best use of molecular docking in getting in-depth idea of the interaction of the selected compounds with their target protein. The binding energy and their docking pose will helps us in selecting some potent inhibitors of Plasmodium falciparum. It will also encourage the researcher to get an idea about structure activity relationship of the various functional group attached to the 4-Amino-7-chloquine clubbed pyridine and other amine analogs and their potency as an antimalarial agents. The present study will be carried out based on the literature available related with the research topic. Therefore, chances of getting breakthrough are more, which may be patented or published in highly, reputed journals.

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